ADDENDUM TO THE ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN FOR NAVAL STATION, TREASURE ISLAND HUNTERS POINT ANNEX SAN FRANCISCO, CALIFORNIA

January 29, 1992

Section 2.2.2 Selection of Control Station Area

On page 2-3, the third bullet is modified as follows: "As a secondary criterion, the area containing sediments of similar physical characteristics as test (HPA) sediments (e.g. grain size). The emphasis on sediment physical characteristics will be on selecting a sediment that is matched primarily to the grain size preference of the organism and secondarily to the grain size of sediments at HPA."

On page 2-3, Section 2.2.2, reverse order of third and fourth bullets

On page 2-3, Section 2.2.2, a sentence is added to the end of the last paragraph which reads "If control sediment is purchased, the supplier will be required to furnish data to support the assertion that the sediments are not toxic".

Section 2.3.3.1 Amphipod Processing and Maintenance

On page 2-5, Section 2.3.3.1, the following sentence is added to the end of paragraph 2: "In order to initiate the amphipod bioassay tests, there will be no greater than 10% mortality in amphipods during the holding period prior to conducting the bioassays".

Section 2.4.1 Surficial Sediment Grab Sampling Procedures

On page 2-6, paragraph 1, the following sentence is added to the end of the paragraph: "All station locations will be reported in longitude and latitude".

On page 2-7, (paragraph 1), delete sentence one and two; replace with the following: "The ten grab sediment samples from random locations within each test station area (Plate 3) and one grab sediment sample from each reference station (Plate 6) will be obtained using a Van Veen grab sampler. The approximate volume of sediment per grab that will be collected by the Van Veen grab is 366 cubic inches".

On page 2-7, (paragraph 3), "A one-liter aliquot of sediment will be removed from the center of the grab at a consistent depth from the sediment water interface; the aliquot will be taken from the center of the grab in a manner to avoid contact with the sides. The sediment will be placed into a 15 liter container for mixing".

On page 2-7, paragraph 3, the second sentence is deleted. In Section 11.2.1.5, the EPA/COE Greenbook recommends using sieved sediments as soon as possible after the macroinvertebrates are removed. For this reason, as well as the reduced possibility of contamination of sediments during sieving procedures conducted in the laboratory as opposed to field sieving, sediment samples to be used in bioassay tests will be sieved in the laboratory. As press sieving for infauna will be conducted at the bioassay laboratory, sediment sieving is discussed in Section 2.6.1.1 and 2.6.1.2.

On page 2-7, delete paragraph 4 and replace with the following: "When the ten representative samples have all been transferred to the 15 liter container, the sediment will be slowly stirred with a stainless steel rod to ensure adequate mixing. The sediment will be mixed until the color and texture are visually homogenized. Samples for physical and chemical analyses will be removed from the container and placed in the appropriate sample container, which will be filled to exclude air or overlying water, for the necessary subsamples. The remaining portion of the composite sample will be transferred to five 2-liter containers which will be completely filled with sediment in order to exclude air from the sample. The sample containers will then be sealed and labeled with the station identification number for use in the bioassay tests. The 2-liter containers will be stored immediately in an ice chest at 2° to 4° C and maintained at that temperature until the sediment is utilized in the bioassays. The amphipod sediment bioassay, modified solid-phase bioassay, and liquid suspended particulate phase bioassay will be initiated as soon as possible upon receipt of the samples by the laboratory, but in any event, the sample will be used in the bioassay within fourteen days of sample collection. Sediment that is not used in the 'first run' of bioassay tests will be stored in the event a 'second run' is necessary (e.g. if control mortality is greater than 10 percent). These sediments will be used within 42 days of sample collection, as per EPA/COE Implementation Manual, should a second run of tests be necessary."

Section 2.4.2 Sediment Core Sampling Procedures

On page 2-8, paragraph 1, the last sentence is changed to read "The location of each core sample station will be recorded using Loran C coordinates and will be reported in longitude and latitude".

On page 2-8, the last paragraph is modified to read "Sediment core samples will be collected from each station (Plate 3) using a 2-inch diameter gravity-type corer deployed from a boat. A minimum core penetration of three feet below the sediment-water interface will be achieved and a minimum of two feet of sediment will be collected from within the core.

On page 2-9, the second paragraph is modified to read "Discrete core samples will be extracted from the bottom 6 inches of the cores at the laboratory to avoid potential sample contamination in the field. The laboratory analytical program for sediment samples is discussed in Section 2.7 and summarized in Table 3."

Section 2.5 Preparation of Seawater for Bioassay Systems

On page 2-9, Section 2.5, paragraph 2 which discusses wet-sieving and static-renewal procedures is deleted.

Section 2.6 BIOASSAY TESTING PROCEDURES

Solid phase bioassays will be conducted with filter-feeding, deposit-feeding and burrowing marine species as specified in the EPA/COE "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual" (Testing Manual), 1991. The amphipod sediment bioassay will be conducted on the amphipod E. estuarius utilizing a modified Swartz et al. (1985) protocol. The solid-phase bioassays conducted on the marine worm; N. caecoides, and the mysid shrimp; H. costata, will utilize solid-phase bioassay protocol outlined in the EPA/COE Testing Manual.

Liquid suspended particulate phase bioassays will be conducted on zooplankton, crustaceans, and fish as specified in the EPA/COE Testing Manual. The liquid suspended particulate phase bioassays conducted with bivalve larvae; C. gigas or M. edulis, the mysid shrimp; H. costata, and the Sanddab; C. stigmaes will utilize the liquid suspended particulate phase bioassay protocol outlined in the EPA/COE Testing Manual.

These bioassay testing procedures are described in detail in Sections 2.6.1.1 through 2.6.5.C.10 of the ESAP and addendum.

Section 2.6.1.1 Sediment Preparation (Amphipod Sediment Bioassay)

On page 2-9 and 2-10, Section 2.6.1.1 in the ESAP is modified to read as follows:

Just prior to initiation of the bioassays (within 48 hours), preparation of the control sediments will be conducted using the following methods:

- Control sediments will be removed from the interior of the control sample container
- During sieving, a separate container will be set up to collect control sediment
- Approximately 250 mL of control sediment will be collected for each bioassay beaker
- Sediment will be press-sieved through a 0.5 mm mesh screen to remove test organisms from the sediment
- The interstitial salinity of the control sediment will be determined by refractometer.

Because the amphipods may be adversely affected by salinity stress if the interstitial water salinity is not within the tolerance limits of the test species, the interstitial salinity may require adjustment. If adjustment of the interstitial water salinity of the control sediment is necessary, the sediment will be resieved using the following methods:

- The control sediment will be resieved through a 0.5 mm mesh screen into water of the bioassay salinity (15 ppt)
- The sediment will be allowed to settle for at least 4 hours, after which the overlying water will be decanted
- The control sediment will be held at 4° C until the bioassay chambers are prepared.

Prior to initiation of the bioassay, preparation of the test and reference sediments will be conducted using the following methods:

- Sediments will be removed from the 2-liter composite sample containers
- Approximately 250 mL of test sediment will be collected for each bioassay beaker

- o Test sediment will be press-sieved through a 0.5 mm mesh screen to remove any macrobenthic organisms that may be present in the sediment

- o Any material remaining on the screen will be discarded

- o The sieved test sediment will be returned to its storage container and held at 4° C until used in the bioassay.

Section 2.6.1.2 Test Chamber Systems

On page 2-10, the second paragraph of Section 2.6.1.2 starting with "Prepared seawater . . . " and continuing to page 2-11, is deleted.

On page 2-10, the following paragraphs are added: "Reference toxicant tests will be utilized to determine 24-hour LC50 values for E. estuarius exposed in clean seawater without sediment to the reference toxicant. The reference toxicant used in the reference toxicant bioassay will consist of reagent-grade cadmium chloride ($CdCl_2$). A geometric dilution series of six unreplicated concentrations and a control will be used. The concentrations used will be as follows: .16 g/L, .31 g/L, .63 g/L, 1.25 g/L, 2.5 g/L, and 5.0 g/L. This concentration range will usually give greater than 50 percent mortality in at least one concentration and less than 50 percent in at least one concentration. Ten organisms will be used per exposure chamber. The reference toxicant tests will be conducted under static conditions.

The reference toxicant tests will otherwise be conducted following the amphipod sediment bioassay methodology with the following exceptions:

- o Section 2.6.1.1 outlining sediment preparation procedures are not necessary as the reference toxicant tests will be conducted without sediment

- o The following procedures will be utilized in the place of those procedures outlined in Section 2.6.1.3:

    a. A concentrated stock solution of known concentration will be prepared using cadmium chloride and uncontaminated control seawater which has been adjusted to test salinity $\pm$ 2 ppt

    b. Test concentrations of reference toxicant will be prepared in a geometric dilution series as described above, based on a test volume equal to that of the seawater and test sediment used in the loaded amphipod sediment bioassay

c. Toxicant treatments will be loaded in the test chambers. Prenumbered toxicant treatment chambers shall be assigned random placement in a temperature controlled room

d. Toxicant treatment chambers will be allowed to equilibrate overnight

- The sediment screening procedure in Section 2.6.1.6 will not be necessary. Instead, tank water will be searched thoroughly for organisms."

Section 2.6.1.3 Introduction of Seawater and Sediments to Test Chambers

On page 2-11, Section 2.6.1.3 is modified, as per Swartz, et al., to read as follows:

On the day before the bioassay, the seawater and sediments will be added to the test chambers by the following procedures:

- Each test sediment sample will be mixed within its storage container and approximately 175 mL of test sediment will be placed in the bottom of each one-liter test chamber to create a 2-cm layer of sediment on the bottom

- The weight of sediment necessary to make a 2-cm deep layer (175 mL) in the first beaker will be added to the test replicate chambers

- The same procedure will be applied to control and reference sediment beakers

- Treatments will be randomly assigned to prenumbered bioassay beakers

- The sediment aliquots will be settled in the beakers by smoothing with a trifluoroethylene resin spoon and bubbles removed by gently tapping the beakers against the palm of the hand

- A disk cut from black plastic sheeting (attached to a nylon string for removal) will be placed on the sediment surface to minimize sediment disruption as bioassay seawater is added

- Bioassay seawater will be added up to the 750 mL mark on each beaker

- o The disk will then be removed and rinsed in bioassay water between beakers and changed between treatments

- o The beakers will be covered with watchglasses, placed in a temperature controlled room, and allowed to equilibrate overnight.

Section 2.6.1.4 Introduction of Organisms to Test Chambers

On page 2-11, Section 2.6.1.4 is modified to read as follows:

On the day of the initiation of the bioassay, the following procedures will be used for the preparation and introduction of amphipods to the test chambers:

- o Amphipods will be removed from the holding sediment using a 0.5 m sieve and transferred to sorting trays

- o Healthy, active organisms will be removed from the sorting tray and sequentially distributed (20 amphipods per fingerbowl) among 10-cm fingerbowls each containing 150 mL of bioassay seawater without sediment

- o The number of amphipods distributed to each fingerbowl will be recounted by transferring them to a separate fingerbowl

- o The amphipods will then be added to the bioassay beakers by placing a black plastic disk on the seawater surface and gently pouring the entire contents of the fingerbowls into the beaker

- o Any adhering amphipods will be removed by washing the fingerbowl with bioassay seawater

- o A total of twenty amphipods will be distributed to each test beaker

- o The seawater level will then be brought up to the 950 mL mark in the beaker with 15 ppt salinity seawater and the disk removed

- o Any amphipods floating on the water surface will be gently submerged with the beaker cover edge

- o After 1 hour, any organisms that have not buried in the sediment will be removed and replaced.

Section 2.6.1.5 Initiation of Amphipod Sediment Bioassay

On page 2-12, the last bullet indicating that ammonia concentrations will be measured daily in the test chambers is deleted. The following sentence is added to the last paragraph in Section 2.6.1.5: "Ammonia concentrations in each test chambers will be measured and recorded at the initiation and completion of the bioassays."

Section 2.6.2.1 Sediment Preparation

On page 2-12, the first bullet is modified as follows: "Test and reference sediments will be removed from the interior of the 2-liter composite sample containers. Control sediments iwll be removed from the control sediment holding container."

On page 2-12, the second bullet is modified as follows: "Control, reference, and test sediments will be press-sieved through a 0.5 mm mesh screen to remove infauna. Sediment will be retained in an uncontaminated container.

Section 2.6.2.3 Test Chamber Systems (Modified Solid-Phase Bioassays)

On page 2-13, Section 2.6.2.3, paragraph 2 is modified to read "Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as water from which the test organisms were collected will be used in the bioassay tests. Salinity will be maintained at $\pm$ 2 ‰ and temperature within $\pm$ 2°C. Dissolved oxygen will be maintained above 40 percent saturation."

On page 2-13, the following paragraphs are added: "Reference toxicant tests will be utilized to determine 24-hour LC50 values for N. caecoides and H. costata exposed in clean seawater without sediment to reference toxicants. The reference toxicant used in the reference toxicant bioassay will consist of reagent-grade cadmium chloride ($CdCl_2$). A geometric dilution series of five unreplicated concentrations and a control will be used for each organism. A concentration range will be selected to give greater than 50 percent mortality in at least one concentration and less than 50 percent mortality in at least one concentration. Ten organisms will be used per exposure chamber. The reference toxicant tests will be conducted under static conditions.

The reference toxicant tests will otherwise be conducted following the modified solid-phase bioassay methodology with the following exceptions:

- o Section 2.6.2.1 outlining sediment preparation procedures are not necessary as the reference toxicant test will be conducted without sediment

- o The following procedures will be utilized in place of the procedures outlined in Section 2.6.2.4:

    a. A concentrated stock solution of known concentration will be prepared using cadmium chloride and uncontaminated control seawater which has been adjusted to test salinity $\pm$ 2 ppt

    b. Test concentration of reference toxicant will be prepared in a geometric dilution series based on a test volume equal to that of the seawater and test sediment used in the loaded solid-phase test

    c. Toxicant treatments shall be loaded in the test chambers. Prenumbered toxicant treatment chambers shall be assigned random placement in temperature controlled rooms

    d. Toxicant treatment chambers will be allowed to equilibrate overnight

- o Sediment screening procedures in Section 2.6.2.6 will not be necessary. Instead, tank water will be searched thoroughly for organisms."

Section 2.6.2.4 Introduction of Seawater and Sediments to Test Chambers

On page 2-13, Section 2.6.2.4, the last bullet reading "Seventy-five percent of seawater volume in the test containers will be replaced using gentle siphoning and addition techniques one hour prior to addition of organisms." is deleted.

Section 2.6.2.6 Initiation of Bioassay

On page 2-14, Section 2.6.2.6, the last bullet reading "Ammonia concentrations in tank water" is deleted. A sentence is added to the following paragraph, that reads "Ammonia concentrations will be measured and recorded at the initiation and completion of the bioassays for each test chamber."

Section 2.6.2.7 Completion of Solid Phase Bioassay

On page 2-14, Section 2.6.2.7, the last sentence of the second paragraph is modified to read "Organisms which are not recovered will be considered dead because once dead, organisms may decompose or be cannibalized".

Section 2.6.3 Presentation of Data

On page 2-15, a paragraph is added to the end of Section 2.6.3 that reads "If control mortality is not acceptable ($\geq$10% mortality), the bioassay tests will be repeated."

Section 2.6.4 Statistical Analysis and Interpretation of Results

On page 2-15, Section 2.6.4, the following sentences are added to the end of paragraph 1. "For solid-phase bioassays, the statistical hypothesis to be tested is that there is no difference in toxicity between the control sample and the test samples. The reference samples in South San Francisco bay will not be used in statistical comparisons against test samples from HPA to define toxic sediments."

Section 2.6.5 Liquid Suspended Particulate Phase Bioassays

Section 2.6.5, page 2-15 through 2-18, is deleted and is replaced with the following Sections 2.6.5, 2.6.5A, 2.6.5B, and 2.65C.

Section 2.6.5.A Liquid Suspended Particulate Phase Bioassays - Mysid Shrimp

2.6.5.A.1 Test Organisms

Mysid shrimp, Holmesimysis costata, of similar size will be purchased for use in the mysid shrimp liquid suspended particulate phase bioassays. Organisms that are damaged during transport and handling will be discarded. Prior to initiation of bioassays, the mysid shrimp will be held in holding tanks with a minimum dissolved oxygen content of 60% saturation or greater. The mysid shrimp will be fed with a concentrated brine shrimp nauplii suspension twice a day during the holding period.

2.6.5.A.2 Organism Preparation

Just prior to initiation of the liquid suspended particulate phase bioassay, the following procedures will be conducted:

- From holding tanks containing seawater, the mysid shrimp will be gently removed in fine-mesh nets. If fine-mesh nets prove insufficient for removal of the mysid shrimp from the holding tanks, large diameter pipets will be used.

- Damage to the organisms will be avoided by handling with extreme care; mysid shrimp which appear damaged or that exhibit abnormal behavior will be discarded

- Specimens of Holmesimysis costata of approximate equal size will be randomly divided into test containers so that each contains 10 individuals.

2.6.5.A.3 Sediment-Water Preparation

Prior to initiation of the liquid suspended particulate phase bioassay, preparation of the sediment-water mixture will be conducted using the following methods:

- One liter sediment subsamples will be removed for each of the composite samples containers

- The sediments will be combined with prepared artificial seawater in a volumetric sediment-to-water ratio of 1:4 at room temperature (22° ± 2° C)

- The sediment-seawater mixture will be thoroughly mixed for 30 minutes

- The mixture will then be allowed to settle for 1 hour

- The liquid and sediment remaining in suspension after 1 hour will be siphoned off, taking care not to disturb the settled material, for immediate use in the bioassay.

2.6.5.A.4 Test Chamber System

Test chambers to be used in the mysid shrimp liquid suspended particulate phase bioassays will have a volume of 5 liters. Five replicate test containers will be used for the control station, the three reference stations and for each of the 17 test stations.

Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as the water from which the test organisms were

collected will be used for the sediment-water mixture. Salinity will be maintained at ± 2 ‰ and temperature at ± 2° C. A dissolved oxygen content of 40% saturation or greater will be maintained throughout the tests.

Three concentrations of test material suspensions will be tested at concentrations of 100, 50, and 10 percent.

Reference toxicant tests will be utilized to determine 24-hour LC50 values for H. costata exposed in clean seawater without sediment to reference toxicants. The reference toxicant used in the reference toxicant bioassay will consist of reagent-grade cadmium chloride ($CdCl_2$). A geometric dilution series of five unreplicated concentrations and a control will be used. A concentration range will be selected to give greater than 50 percent mortality in at least one concentration and less than 50 percent mortality in at least one concentration. Ten organisms will be used per exposure chamber. The reference toxicant tests will be conducted under static conditions.

The reference toxicant tests will otherwise be conducted following the liquid suspended particulate phase bioassay methodology for mysid shrimp with the following exceptions:

- Section 2.6.5.A.3 outlining sediment-water preparation are not necessary as the reference toxicant will be conducted without sediment

- The following procedures will be utilized in place of the methods outlined in Section 2.6.5.A.5:

    a. A concentrated stock solution of known concentration will be prepared using cadmium chloride and uncontaminated control seawater which has been adjusted to test salinity ± 2 ppt

    b. Test concentrations of reference toxicant shall be prepared in a geometric dilution series based on a test volume equal to that of the seawater and test sediment used in the liquid suspended particulate phase bioassay for mysid shrimp

    c. Toxicant treatments will be loaded in the test chambers. Prenumbered toxicant treatment chambers shall be assigned random placement in a temperature controlled room

    d. Toxicant treatment chambers will be allowed to equilibrate overnight.

2.6.5.A.5 Introduction of Seawater-Sediment Mixture to Test Chambers

The 1:4 sediment-water mixture will be introduced to the test chambers immediately upon completion of the sediment/water preparation procedures described in Section 2.6.5.3.

2.6.5.A.6 Introduction of Organisms to Test Chambers

Following preparation and selection of individual mysid shrimps for use in the bioassay, the organisms will be released to the chambers.

2.6.5.A.7 Initiation of Liquid Suspended Particulate Phase Bioassay

The bioassay will begin with the introduction of the mysid shrimp to the test tanks. The test duration will be 96 hours.

At 0, 4, 24, 48, 72, and 96 hours, the number of live mysid shrimp will be recorded. An organism will be considered dead if it does not respond to the probing of a sensitive body part and will be removed from the test chamber. In addition, any behavioral abnormalities exhibited by test organisms will be recorded. At each observation period, dead organisms, molted exoskeletons, and food debris will be removed from the test chambers by pipette or forceps.

Daily levels of the following water parameters will be measured and recorded:

- Salinity of test water
- Temperature of test water
- Dissolved oxygen content of test water
- pH of test water.

The tank water will be aerated only when necessary to maintain the dissolved oxygen content above 40% saturation.

2.6.5.A.8 Completion of Bioassay

After 96 hours, the tank water containing the mysid shrimp will be searched thoroughly for organisms. The organisms will be considered alive if they show any response to gentle probing or gentle swirling of the water. The number of live organisms will be counted and recorded.

2.6.5.A.9 Presentation of Data

If control mortality is greater than 10 percent, the results of the bioassay may not be evaluated. In this case, species selection and other test variables will be reevaluated in an attempt to reduce unacceptably high control mortality and the tests will be repeated. If control mortality is less than 10 percent, the bioassay data will be presented in tabular form and will contain the following information:

- o Scientific name of test species
- o Number of organisms in each treatment at test start
- o Number of organisms alive at each observation period
- o Number of organisms recovered alive at test end
- o Any behavioral abnormalities recorded.

2.6.5.A.10 Statistical Analysis and Interpretation of Results

If control mortality is less than 10 percent and is less than the mortality in the test material treatment, the test data will be statistically analyzed to determine if there is a significant difference in survival between control and test samples. The t-test (Snedecor and Cochran, 1980) will be used to compare the mean control and test survivals following the Levine's test for the homogeneity of sample variances. In the event that the liquid suspended particulate phase bioassay data fail to show homogeneity of variances, the t-test for unequal variances (Snedecor and Cochran, 1980) will be used.

If mortality in the test material exceeds 50 percent, an LC50 value (lethal concentration to 50 percent of the test organisms) will be calculated for any dilutions in which greater than 50 percent mortality occurs.

In the event that no mortality occurs in either control or test tanks, or that survival of organisms in the test tanks is equal to or greater than control organism survival, no statistical analyses will be performed.

2.6.5.B Liquid Suspended Particulate Bioassay - Bivalve Larvae

2.6.5.B.1 Test Organisms

Upon purchase, adults (Crassostrea gigas or Mytilus edulis will be transported without delay to the laboratory, cleaned of detritus and fouling organisms

such as barnacles, and placed in flowing water with a salinity and temperature suitable to the species (± 2‰ and ± 2°C that of the collection or culture water). Adults that are injured during handling will be discarded.

The brood stock will be gradually conditioned to the test temperature and salinity. Temperature will be changed at a rate not to exceed 2° C/day and salinity at a rate not to exceed 5 g/L/day salts. The concentration of dissolved oxygen will be maintained between 60 to 100% of saturation. The adults will be provided with cultivated phytoplankton to prevent malnutrition during the conditioning and holding period. The brood stock will be carefully observed daily during holding and conditioning for signs of stress and mortality. Dead bivalves and gaping mollusks that do not close when touched with a probe will be discarded daily.

Embryos used in the bioassay will be obtained from females and males that have been maintained for at least two weeks in the dilution water in the laboratory before they are induced to spawn.

2.6.5.B.2 Organism Preparation

Prior to initiation of the bivalve larvae bioassay, the following procedures will be conducted:

- In preparation for thermal stimulation of spawning, 10 to 50 animals will be selected from a population of bivalves with ripe gonads and placed in small groups in spawning chambers (Pyrex dishes)
- Chambers will be filled with dilution water at the conditioning temperature, and the animals will be allowed to begin pumping before starting thermal stimulation
- The spawning chambers will be placed in a water bath filled with hot water
- When the temperature in the spawning chamber attains a temperature of 5 to 10° C above the conditioning temperature, the water bath will be drained
- Females will be additionally stimulated to spawn by the addition of sperm from a sacrificed or naturally spawned male
- The spawning animal will be left in the chamber until the release of gametes ceases, at which time it will be returned to a holding tank

- Eggs will be passed through a 75 um screen and the concentration of eggs determined by counting a sample of the egg suspension. The egg density will be adjusted to the range 20 to 50 eggs/mL before adding sperm

- After sperm have been verified by microscopic examination, the sperm suspension will be passed through a 37-um screen to remove feces and other extraneous material

- Within one hour of spawning, eggs and sperm will be combined in a one liter Nalgene beaker

- Fertilization will be accomplished at the spawning temperature and the suspension held at that temperature until it is determined that fertilization has been accomplished

- After the eggs have been fertilized, the embryo suspension will be poured through a 54-um screen to remove debris. Excess sperm, small protozoa, and bacteria will be removed by pouring the embryos onto a 22-um screen, washing with dilution water and backwashing into a suitable container filled with filtered seawater at incubating temperature

- Embryos will be agitated using a perforated plunger to keep them in suspension and will be utilized in the bioassay within 2 hours of fertilization.

2.6.5.B.3 Sediment-Water Preparation

Prior to initiation of the liquid suspended particulate phase bioassay, preparation of the sediment-water mixture will be conducted using the following methods:

- One liter sediment subsamples will be removed for each of the composite sample containers

- The sediments will be combined with prepared artificial seawater in a volumetric sediment-to-water ration of 1:4 at room temperature

- The sediment-seawater mixture will be thoroughly mixed for 30 minutes

- The mixture will then be allowed to settle for 1 hour

- o The liquid and sediment remaining in suspension after 1 hour will be siphoned off, taking care not to disturb the settled material, for immediate use in the bioassay

- o The temperature of the test media will be adjusted to the appropriate temperature for the organism embryo tested (C. gigas - 20° C, M. edulis - 15° C).

2.6.5.B.4 Test Chamber System

Test containers to be used in the liquid suspended particulate phase bioassays for the bivalve larvae will have a volume of 1 liter. Five replicate test containers will be used for the control station, the three reference stations and for each of the 17 test stations.

Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as the water from which the test organisms were collected will be used for the sediment-water mixture. Salinity will be maintained at ± 2 ‰ and temperature at ± 2° C.

Three concentrations of test material suspension will be tested at concentrations of 100, 50, and 10 percent.

Reference toxicant tests will be conducted to determine 24 hour LC50 and EC50 values for bivalve larvae exposed to reference toxicants in clean, unfiltered seawater without sediment. The reference toxicant used will consist of reagent-grade cadmium chloride ($CdCl_2$). The reference toxicant bioassay will use geometric dilution series of seven unreplicated concentrations and a control. The concentrations used will be as follows: 3.2 g/L, 5.6 g/L, 10 g/L, 18 g/L, 32 g/L, 56 g/L, and 100 g/L. This concentration range gives greater than 50 percent mortality in at least one concentration and less than 50 percent mortality in at least one concentration. Ten organisms will be used per exposure chamber. The reference toxicant tests will be conducted under static conditions.

The reference toxicant tests will otherwise be conducted following the liquid suspended particulate phase bioassay (bivalve larvae) methodology with the following exceptions:

- o Section 2.6.5.B.3 outlining sediment-water preparation are not necessary as the reference toxicant test will be conducted without sediment

- The following procedures will be utilized in place of the methods described in Section 2.6.5.B.5:

  a. A concentrated stock solution of known concentration will be prepared using cadmium chloride and uncontaminated control seawater which has been adjusted to test salinity $\pm$ 2 ppt

  b. Test concentration of reference toxicant shall be prepared in a geometric dilution series as described above based on a test volume equal to that of the seawater and test sediment used in the loaded liquid suspended particulate phase bioassay for bivalve larvae

  c. Toxicant treatments will be loaded in the test chambers. Prenumbered toxicant treatment chambers will be assigned random placement in temperature controlled rooms

  d. Toxicant treatment chambers will be allowed to equilibrate overnight to the appropriate temperature for the organism embryo test (C. gigas - 20° C, M. edulis - 15° C)

2.6.5.B.5 Introduction of Seawater-Sediment Mixture to Test Chambers

The 1:4 sediment-water mixture will be introduced to the test chambers immediately upon completion of the sediment/water preparation procedures.

2.6.5.B.6 Introduction of Organisms to Test Chambers

About 1 hour after adding the sperm suspension to the egg suspension, the concentration of embryos in the embryo suspension will be determined by mixing the solution with a perforate plunger, withdrawing a 1-mL sample, placing it in a Sedgwick-Rafter cell, and counting the number of embryos that have developed to the 2-cell stage or beyond. The concentration of embryos in the test solution will be between 15 and 30 embryos per mL.

Concentrations of up to 100 embryos per milliliter do not impair normal development of Crassostrea gigas. Mytilus edulis will develop abnormally at concentrations above 30 embyros/mL.

Within 4 hours after fertilization, equal volumes of the homogeneously mixed embryo suspension will be placed in each test container, which already contains the test solution, in a random order by using a pipet.

2.6.5.B.7 Initiation of Liquid Suspended Particulate Phase Bioassay

The bioassay will begin with the introduction of organisms to the test containers. The test duration will be 48 hours. The organisms will not be fed during the tests.

Levels of the following water parameters will be measured at the beginning and end of each test and recorded:

- o Salinity of the test container water
- o pH of the test container water
- o Dissolved oxygen content of the test container water
- o Daily maximum and minimum temperature of the test container water.

The test container water will not be aerated during the test as bubbles can collect within the mantle cavity of the larvae, and adversely affect larval survival and development.

2.6.5.B.8 Completion of Bioassay

Forty eight hours after beginning the test, the solution in each test chamber will be carefully mixed and preserved in a 5 percent buffered formalin solution. The embryos and larvae will then be placed in a Sedgwick-Rafter counting chamber.

All embryos exhibiting cell division will be counted. Percent abnormality will be determined by enumerating normal and abnormal larvae. All larvae with completely developed shells containing meat will be counted as normal. Empty shells, even if they are completely developed, will not be counted as the larvae are not alive at the end of the test. Larvae with incompletely developed shells after 48 hours may be morphologically normal, but the retarded development is considered likely to reduce their survival in the natural environment. Percent survival will be determined as the number of larvae surviving in each test container relative to the seawater control.

2.6.5.B.9 Presentation of Data

If control mortality is greater than 20 percent, the results of the bioassay may not be evaluated. In this case, species selection and other test variables will be reevaluated in an attempt to reduce unacceptably high control mortality and the tests repeated. If control mortality is less than 20 percent, the

bioassay data will be presented in tabular form and will contain the following information:

- o Scientific name of test species
- o Water quality data measured at the beginning and end of testing (salinity, temperature, dissolved oxygen content, and pH)
- o Individual replicate, and mean and standard deviation data for larval survival after 48 hours
- o Individual replicate, and mean and standard deviation data for larval abnormalities after 48 hours
- o 24 hour LC50 and EC 50 values with reference toxicants.

2.5.6.B.10 Statistical Analysis and Interpretation of Results

If control mortality is less than 20 percent and is less than the mortality in the test material treatment, the test data will be statistically analyzed to determine if there is a significant difference in survival between control and test samples. The t-test (Sndecor and Cochran, 1980) will be used to compare the mean control and test survivals following the Levine's test for the homogeneity of sample variances. In the event that the liquid suspended particulate phase bioassay data fail to show homogeneity of variances, the t-test for unequal variances (Snedecor and Cochran, 1980) will be used.

LC50 value (lethal concentration to 50 percent of the test animals) will be calculated for any dilutions in which greater than 50 percent mortality occurs.

In the event that no mortality occurs in either control or test tanks, or that survival of organisms in the test containers is equal to or greater than control organism survival, no statistical analyses will be performed.

2.6.5.C Liquid Suspended Particulate Phase Bioassay - Sanddab

2.6.5.C.1 Test Organisms

Juvenile sanddabs (Citharichthys stigmaeus) less than 90 days old and of similar size will be purchased from a commercial supplier of wild collected animals for use in the liquid suspended particulate phase bioassay. The organisms will be inspected to assure that they are in good condition and free from disease. Fish that are injured during handling or that appear diseased

will be discarded. To assure healthy test organisms, the fish will be held in the laboratory for a minimum two-day acclimation period.

Loading of fish in holding tanks will not exceed one g/L to avoid overcrowding. The dissolved oxygen content will be maintained by aeration at a minimum of 40% saturation during holding and acclimation periods. Fish will be fed adlibitum at least once a day with brine shrimp during the holding period up to two days before the bioassay test begins. Excess food and fecal material will be removed from the bottom of the tanks. Organisms will be observed carefully each day for signs of disease, stress, physical damage, and mortality. Dead and abnormal specimens will be removed as soon as observed. If greater than 10 percent of the total population becomes infected, the holding tank population will be destroyed.

If the water in which the fish are received differs from test water conditions, they will be gradually conditioned to the test temperature and salinity. Temperature will be changed at a rate not to exceed 2° C/day and salinity at a rate not to exceed 5 g/L/day salts.

2.6.5.C.2 Organism Preparation

Just prior to initiation of the liquid suspended particulate phase bioassay, the following procedures will be conducted:

- o From holding tanks containing seawater, the fish will be gently removed and transferred in fine-mesh nets
- o Damage to the organisms will be avoided by handling with extreme care; organisms which appear damaged or that exhibit abnormal behavior will be discarded
- o Juvenile sanddabs of approximate equal size will be randomly divided into test containers so that each contains 10 individuals.

2.6.5.C.3 Sediment-Water Preparation

Prior to initiation of the liquid suspended particulate phase bioassay, preparation of the sediment-water mixture will be conducted using the following methods:

- o One liter sediment subsamples will be removed for each of the composite sample containers

- The sediments will be combined with prepared artificial seawater in a volumetric sediment-to-water ratio of 1:4 at room temperature (22° ± 2° C)

- The sediment-seawater mixture will be thoroughly mixed for 30 minutes

- The mixture will then be allowed to settle for 1 hour

- The liquid and sediment remaining in suspension after 1 hour will be siphoned off, taking care not to disturb the settled material, for immediate use in the bioassay.

- The temperature of the test media will be adjusted to the appropriate test temperature (20° ± 1° C)

2.6.5.C.4 Test Chamber System

Tanks to be used in the sanddab liquid suspended particulate phase bioassays will have a volume of at least 10 liters. Five replicate tanks will be used for the control station, the three reference stations and for each of the 17 test stations.

Prepared seawater of approximately the same temperature, salinity and dissolved oxygen content as the water from which the test organisms were collected will be used for the sediment-water mixture. Salinity will be maintained at ± 2 ‰ and temperature at ± 2° C. A dissolved oxygen content of 40 percent or greater will be maintained throughout the tests.

Three concentrations of test material suspension will be tested at concentrations of 100, 50, and 10 percent.

Reference toxicant tests will be used to determine 24 hour LC50 and EC50 values for sanddabs exposed to reference toxicants in clean, unfiltered seawater without sediment. The reference toxicant used will consist of reagent-grade cadmium chloride ($CdCl_2$). The reference toxicant bioassay will use a geometric dilution series of five unreplicated concentrations and a control. A concentration range will be selected to give greater than 50 percent mortality in at least one concentration and less than 50 percent mortality in at least one concentration. Ten organisms will be used per exposure chamber. The reference toxicant tests will be conducted under static conditions.

The reference toxicant tests will otherwise be conducted following the liquid suspended particulate phase bioassay methodology for sanddabs with the following exceptions:

- Section 2.6.5.C.3 outlining sediment-water preparation procedures are not necessary as the reference toxicant tests will be conducted without sediment

- The following procedures will be utilized in place of the methods described in Section 2.6.5.C.5:

  a. A concentrated stock solution of known concentration will be prepared using cadmium chloride and uncontaminated control seawater which has be adjusted to test salinity $\pm$ 2 ppt

  b. Test concentrations of the reference toxicant will be prepared in a geometric dilution series based on a test volume equal to that of the seawater and test sediment used in the loaded liquid suspended particulate phase bioassay for sanddabs

  c. Toxicant treatments will be loaded in the test chambers. Prenumbered toxicant treatment chambers will be assigned random placement in a temperature controlled room

  d. Toxicant treatment chambers will be allowed to equilibrate overnight

2.6.5.C.5 Introduction of Seawater-Sediment Mixture to Test Tanks

The 1:4 sediment-water mixture will be introduced to the test tanks immediately upon completion of the sediment/water preparation procedures described in Section 2.6.5.3B.

2.6.5.C.6 Introduction of Organisms to Test Tanks

Following preparation and selection of individual fish for use in the bioassay, the fish will be released to the tanks.

2.6.5.C.7 Initiation of Liquid Suspended Particulate Phase Bioassay

The bioassay will begin with the introduction of the fish to the test tanks. The test duration for the sanddab will be 96 hours.

At 0, 4, 24, 72 and 96 hours, the number of live fish will be recorded. A fish will be considered dead if it does not respond to prodding and will be removed from the test tank. In addition, any behavioral abnormalities exhibited by test organisms will be recorded. At each observation period, dead fish will be removed from the tanks.

Daily levels of the following water parameters will be measured and recorded:

- Salinity of tank water
- Temperature of tank water
- Dissolved oxygen content of tank water
- pH of tank water.

The tank water will be aerated only when necessary to maintain the dissolved oxygen content above 60% saturation. The fish will not be fed for the duration of the test. A photoperiod of 16 hours light/8 hours dark will be maintained during the test.

2.6.5.C.8 Completion of Bioassay

After 96 hours, the tank water containing the sanddabs will be searched thoroughly for organisms. The fish will be considered alive if they show any response to prodding or gently swirling of the water. The number of live organisms will be counted and recorded.

2.6.5.C.9 Presentation of Data

If control mortality is greater than 10 percent, the results of the bioassay may not be evaluated. In this case, species selection and other test variables will be reevaluated in an attempt to reduce unacceptably high control mortality and the tests repeated. If control mortality is less than 10 percent, the bioassay data will be presented in tabular form and will contain the following information:

- Scientific name of test species
- Number of fish in each treatment at test start
- Number of fish alive at each observation period
- Number of fish recovered alive at test end

- o Any behavioral abnormalities recorded.

2.6.5.C.10 Statistical Analysis and Interpretation of Results

If control mortality is less than 10 percent and is less than the mortality in the test material treatment, the test data will be statistically analyzed to determine if there is a significant difference in survival between control and test samples. The t-test (Snedecor and Cochran, 1980) will be used to compare the mean control and test survivals following the Levine's test for the homogeneity of sample variances. In the event that the liquid suspended particulate phase bioassay data fail to show homogeneity of variances, the t-test for unequal variances (Snedecor and Cochran, 1980) will be used.

If mortality in the test material exceeds 50 percent, an LC50 value (lethal concentration to 50 percent of the test organisms) will be calculated for any dilutions in which greater than 50 percent mortality occurs.

In the event that no mortality occurs in either control or test tanks, or that survival of organisms in the test tanks is equal to or greater than control organism survival, no statistical analyses will be performed.

3.0 TASK 2 - EVALUATION OF WHETHER PERSISTENT AND BIOACCUMULATIVE SUBSTANCES MAY BE ENTERING THE SAN FRANCISCO BAY FROM HPA

Section 3.5 Collection of Mussels from Uncontaminated Area

On page 3-3, Section 3.5, paragraph 1, sentence 3 is modified to read "The mussel shell length and mussel weight will be measured and recorded upon collection for size requirement verification and for later determination of growth following mussel deployment".

Section 3.7 Retrieval and Storage of Transplanted Mussels

On page 3-6, Section 3.7, a paragraph is added following paragraph 2 which reads "Mussel shell length and weight will be measured and recorded upon retrieval for comparison with mussel length and weight information obtained upon initial collection to determine mussel growth during the deployment period".

4.0 TASK 3 - EVALUATION OF STORM WATER RUNOFF TOXICITY

Section 4.4.1 Collection of Composite Storm Water Runoff Samples

On page 4-3 and 4-4, Section 4.4.1, paragraph 2, a sentence is inserted between the fourth and fifth sentence that reads "Storm water will be collected until cessation of runoff or until 8 hours elapsed time, whichever occurs first. Storm water collection will also cease if flow reversal, which will be used as a general indicator of tidal backflow, occurs in the storm drain."

On page 4-4, Section 4.4.1, paragraph 3, the last sentence is modified to read "Storm water samples will also be submitted for chemical analysis for CLP metals, CLP VOCs, CLP SOCs, CLP pesticides and PCBs, tributyltin by GC/FID, and for analysis of fecal coliforms.

On page 4-4, the following information is included at the end of Section 4.4.1:

Approximately 100 mL of storm water will be collected at each storm water station using dedicated, sterilized, stainless-steel bailers for use in the fecal coliform tests. One discrete sample will be taken at each storm water sampling point. The samples will be placed in 100 mL sterilized, wide-mouth, glass containers. At least 2.5 cm (1 inch) of air space will be left in the sample bottle to facilitate mixing of the sample by shaking. Care will be taken to avoid contamination of the sample at the time of collection or in the period before examination. During sampling, the cap and neck of the sample bottle will not be handled to avoid contamination. The sample bottle will be held near the base, filled without rinsing, the cap replaced, and the container immmediately sealed. The sample bottles will be labeled, placed in cooler maintained at 4° C, and transported to the laboratory when sampling is complete. Samples may be held for a maximum of 30 hours.

Section 4.6.2 Preparation

On page 4-6, Section 4.6.2, the fourth bullet is changed to read "Sample water will be filtered with a plankton net to remove organisms."

Section 4.10

The Quality Assurance Summary information previously contained in Section 4.10, is now Section 4.11. Section 4.10 now contains biological analysis information as follows:

Section 4.10 Biological Analysis

Analysis for fecal coliforms will be conducted on storm water runoff samples from each storm water test station to provide information regarding the potential presence of human waste in the storm water, to be used as a screening tool to assess the possible interconnection of storm water and sewer lines at Hunter's Point Annex. Collection, preservation and storage methods for the samples used for analyses are described in Section 4.4.

The storm water samples will be analyzed for fecal coliforms using the fecal coliform membrane filter procedure. The procedure will be performed in accordance with the 909C methods outlined in "Standard Methods for the Examination of Water and Wastewater", 17th Edition, 1989. Samples will be sent to an analytical laboratory immediately following collection. The laboratory will be certified by the California Department of Health Services.

Section 4.11 Quality Assurance Summary

The Quality Assurance Summary information previously labeled as Section 4.10 in the ESAP, is now Section 4.11. The information remains the same.

Table 3. Sampling and Analytical Program
Page 1 of 2

Evaluation Program and Sample Location Numbers	Number of Samples[a]	Media Type[b]	Radio-Activity Screen	Toxicity Testing	Physical Testing[c]	Radio-Activity Testing[d]	Total Organic Carbon	In-organics/ Metals	Pesti-cides/ PCBs	Semi-Volatile Organics	Tribu-tyltin[e]	Volatile Organics
Sediment Toxicity												
S-1 to S-17	17	S	X	X[f]	X	X	X	X	X	X	X	--
Reference	3	S	X	X[f]	X	X	X	X	X	X	X	--
Control	1	S	X	X[f]	X	--	--	--	--	--	--	--
Sediment Cores	19	S	X	--	X	X	X	X	X	X	X	X
Bioaccumul -ative Effect												
M-1 to M-17	17	T	X	--	--	X	--	X	X	X	X	--
Background	1	T	X	--	--	--	--	X	X	X	X	--
Reference	2	T	X	--	--	--	--	X	X	X	X	--
Storm Water Toxicity												
ST1 to ST4	4	SW	--	X[g]	--	--	--	X	X	X	X	X
B-1 to B-4	4	BW	--	X[g]	--	--	--	X	X	X	X	X
Reference	1	BW	--	X[g]	--	--	--	--	--	--	--	--

a These numbers describe composited samples. The samples will be sub-sampled for screening of radioactivity, toxicity testing, physical testing, chemical analyses, or field and laboratory Quality Control (QC) samples
b Media Type: S = sediment, T = tissue, SW = storm water, BW = bay water
c Physical testing includes determination of grain size by ASTM Method D422
d Laboratory testing of radioactivity will be conducted on samples exhibiting radioactivity above background levels as determined by radioactivity screening. Radioactivity screening will include measurement of alpha and beta particles and gamma rays
e Analytical method: n-Pentyl Derivitization with Gas Chromatography/Flame Photometric Detection
f Toxicity testing of sediment samples involves the use of five replicates in 10-day solid phase bioassays and liquid suspended particulate phase bioassays
g Toxicity testing of storm and bay water samples involves a five dilution series
h Fecal coliform testing will be conducted by Fecal Coliform Filter Membrane Procedure (Standard Method 909C)

Table 3. Sampling and Analytical Program

Evaluation Program and Sample Location Numbers	Number of Samples[a]	Media Type[b]	Fecal Coliform Testing
Sediment Toxicity			
S-1 to S-17	17	S	---
Reference	3	S	---
Control	1	S	---
Sediment Cores	19	S	---
Bioaccumulative Effect			
M-1 to M-17	17	T	---
Background	1	T	---
Reference	2	T	---
Storm Water Toxicity			
ST1 to ST4	4	SW	X[h]
B-1 to B-4	4	BW	---
Reference	1	BW	---

a These numbers describe composited samples. The samples will be sub-sampled for screening of radioactivity, toxicity testing, physical testing, chemical analyses, or field and laboratory Quality Control (QC) samples
b Media Type: S = sediment, T = tissue, SW = storm water, BW = bay water
c Physical testing includes determination of grain size by ASTM Method D422
d Laboratory testing of radioactivity will be conducted on samples exhibiting radioactivity above background levels as determined by radioactivity screening. Radioactivity screening will include measurement of alpha and beta particles and gamma rays
e Analytical method: n-Pentyl Derivitization with Gas Chromatography/Flame Photometric Detection
f Toxicity testing of sediment samples involves the use of five replicates in 10-day solid phase bioassays and liquid suspended particulate phase bioassays
g Toxicity testing of storm and bay water samples involves a five dilution series
h Fecal coliform testing will be conducted by Fecal Coliform Filter Membrane Procedure (Standard Method 909C)

Table 7. Analytical Methods for Storm Water Analyses

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
ST-1 - ST-4 B-1 - B-4	Water	CLP Inorganics	Aluminum	200.0
			Antimony	3.0
			Arsenic	10
			Barium	100.0
			Beryllium	5.0
			Cadmium	5.0
			Calcium	1000
			Chromium (total)	10.0
			Cobalt	50.0
			Copper	25
			Iron	100
			Lead (total)	3.0
			Magnesium	1000
			Manganese	15.0
			Mercury	0.5
			Molybdenum	10.0
			Nickel	40.0
			Potassium	1000
			Selenium	5.0
			Silver	10.0
			Sodium	1000
			Thallium	10.0
			Tin	40.0
			Vanadium	50.0
			Zinc	20.0
		CLP Pesticides/PCBs	alpha-BHC	0.05
			beta-BHC	0.05
			gamma-BHC (Lindane)	0.05
			delta-BHC	0.05
			Heptachlor	0.05
			Aldrin	0.05

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			Heptachlor epoxide	0.05
			Endosulfan I	0.1
			p,p'-DDE	0.1
			Dieldrin	0.1
			Endrin	0.1
			p,p'-DDD	0.1
			Endosulfan II	0.1
			p,p'-DDT	0.1
			Endrin aldehyde	0.1
			Endosulfan sulfate	0.1
			p,p'-Methoxychlor	0.5
			Endrin ketone	0.1
			Technical chlordane	0.5
			Toxaphene	1.0
			Aroclor 1016	0.5
			Aroclor 1221	0.5
			Aroclor 1232	0.5
			Aroclor 1242	0.5
			Aroclor 1248	0.5
			Aroclor 1254	1.0
			Aroclor 1260	1.0
		CLP SOCs	Phenol	10
			bis(2-Chloroethyl) Ether	10
			2-Chlorophenol	10
			1,3-Dichlorobenzene	10
			1,4-Dichlorobenzene	10
			Benzyl Alcohol	10
			1,2-Dichlorobenzene	10
			2-Methylphenol	10
			bis(2-Chloroisopropyl) Ether	10
			4-Methylphenol	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			N-Nitroso-di-n-Propylamine	10
			Hexachloroethane	10
			Nitrobenzene	10
			Isophorone	10
			2-Nitrophenol	10
			2,4-Dimethylphenol	10
			Benzoic Acid	50
			bis(2-Chloroethoxy)Methane	10
			2,4-Dichlorophenol	10
			1,2,4-Trichlorobenzene	10
			Naphthalene	10
			4-Chloroaniline	10
			Hexachlorobutadiene	10
			4-Chloro-3-Methylphenol	10
			2-Methylnaphthalene	10
			Hexachlorocyclopentadiene	10
			2,4,6-Trichlorophenol	10
			2,4,5-Trichlorophenol	50
			2-Chloronaphthalene	10
			2-Nitroaniline	50
			Dimethylphthalate	10
			Acenaphthylene	10
			3-Nitroaniline	50
			Acenaphthene	10
			2,4-Dinitrophenol	50
			4-Nitrophenol	50
			Dibenzofuran	10
			2,4-Dinitrotoluene	10
			2,6-Dinitrotoluene	10
			Diethylphthalate	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			4-Chlorophenyl-Phenyl Ether	10
			Fluorene	10
			4-Nitroaniline	50
			4,6-Dinitro-2-Methylphenol	10
			N-Nitrosodiphenylamine	10
			Azobenzene	10
			4-Bromophenyl-Phenyl Ether	10
			Hexachlorobenzene	10
			Pentachlorophenol	50
			Phenanthrene	10
			Anthracene	10
			Di-n-Butylphthalate	10
			Fluoranthene	10
			Benzidine	50
			Pyrene	10
			Butylbenzylphthalate	10
			3,3'-Dichlorobenzidine	20
			Benzo(a)Anthracene	10
			bis(2-Ethylhexl)phthalate	10
			Chrysene	10
			Di-n-Octylphthalate	10
			Benzo(b)Fluoranthene	10
			Benzo(k)Fluoranthene	10
			Benzo(a)Pyrene	10
			Indeno(1,2,3-cd)Pyrene	10
			Dibenz(a,h)Anthracene	10
			Benzo(g,h,i)Perylene	10
		GC/FPD[a] with n-pentyl-derivitization	Tributyltin	10

Table 7. Analytical Methods for Storm Water Analyses

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
ST1-ST4	Water	CLP VOCs	Chloromethane	10
			Vinyl Chloride	10
			Bromomethane	10
			Chloroethane	10
			Trichlorofluoromethane	5
			1,1-Dichloroethene	5
			Trichlorotrifluoroethane	5
			Acetone	20
			Carbondisulfide	5
			Methylene Chloride	5
			trans-1,2-Dichloroethene	5
			1,1-Dichloroethane	5
			2-Butanone	20
			cis-1,2-Dichloroethene	5
			Chloroform	5
			1,1,1-Trichloroethane	5
			Carbon Tetrachloride	5
			Benzene	5
			1,2-Dichloroethane	5
			Trichloroethene	5
			1,2-Dichloropropane	5
			Bromodichloromethane	5
			2-Chloroethylvinyl Ether	5
			Vinyl Acetate	10
			trans-1,3-Dichloropropene	5
			4-Methyl-2-Pentanone	10
			Toluene	5
			cis-1,3-Dichloropropene	5
			1,1,2-Trichloroethane	5
			Tetrachloroethene	5
			2-Hexanone	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			Dibromochloromethane	5
			Chlorobenzene	5
			Ethylbenzene	5
			Total Xylenes	5
			Styrene	5
			Bromoform	5
			1,1,2,2-Tetrachloroethane	5
			1,3-Dichlorobenzene	5
			1,4-Dichlorobenzene	5
			1,2-Dichlorobenzene	5
ST1-ST4	Water	Fecal Coliform Filter Membrane Procedure	Fecal Coliforms	NA

a. Gas chromatography/flame photometric detection
NA - Not applicable

ADDENDUM TO THE QUALITY ASSURANCE PROJECT PLAN FOR THE ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN FOR NAVAL STATION, TREASURE ISLAND HUNTERS POINT ANNEX SAN FRANCISCO, CALIFORNIA

January 29, 1992

Section 8.0 STORM WATER TOXICITY SAMPLING PROCEDURES

On page 10, Section 8.0, paragraph 2 of the Quality Assurance Project Plan (QAPjP), the following sentences are inserted between the fourth and fifth sentences: "Storm water will be collected until cessation of runoff or until 8 hours elapsed time, whichever occurs first. Storm water collection will also cease if flow reversal, which will be used as a general indicator of tidal backflow, occurs in the storm drain."

On page 10, Section 8.0, the following paragraphs are inserted between the second and third paragraph: "Approximately 100 mL of storm water will be collected at each storm water station, using dedicated, sterilized, stainless-steel bailers, for use in the fecal coliform tests. The samples will be placed in 100 mL sterilized, wide-mouth glass containers. The sample containers will then be sealed, labeled, placed in coolers at 4° C and transported to the laboratory for incubation and analyses. The samples will be analyzed within 30 hours of collection.

The storm water samples will be analyzed for fecal coliforms using the fecal coliform filter membrane procedure (909C) outlined in "Standard Methods for the Examination of Water and Wastewater", 17th Edition, 1989."

Section 10.1 Equipment Decontamination Procedures

On page 11, Section 10.1 of the QAPjP, the following sentence is inserted following the first paragraph: "The stainless-steel bailers used to collect storm water samples for fecal coliform analysis will be sterilized in the laboratory and carefully wrapped before being taken into the field."

Section 11.5 Sample Handling and Storage

On page 14, Section 11.5 of the QAPjP, the following paragraph is inserted after the first paragraph: "The storm water samples collected for fecal coliform analysis will be collected in 100 mL sterilized wide-mouth glass

containers which will be sealed and stored in a cooler maintained at 4° C until analyzed. The samples will be analyzed within 30 hours of collection."

Section 13.0 ANALYTICAL PROCEDURES

On page 15, Section 13.0 of the QAPjP, a sentence is inserted between the third and fourth sentences of paragraph 2 that reads "Storm water samples will also undergo analyses for fecal coliforms."

TABLES

Tables 1 and 5 have been revised to include fecal coliform testing. Table 2 has been revised to include sample handling information for storm water samples to be analyzed for fecal coliforms.

Table 1. Sampling and Analytical Program
Page 1 of 2

Evaluation Program and Sample Location Numbers	Number of Samples[a]	Media Type[b]	Radio-Activity Screen	Toxicity Testing	Physical Testing[c]	Radio-Activity Testing[d]	Total Organic Carbon	In-organics/ Metals	Pesti-cides/ PCBs	Semi-Volatile Organics	Tribu-tyltin[e]	Volatile Organics
Sediment Toxicity												
S-1 to S-17	17	S	X	X[f]	X	X	X	X	X	X	X	--
Reference	3	S	X	X[f]	X	X	X	X	X	X	X	--
Control	1	S	X	X[f]	X	--	--	--	--	--	--	--
Sediment Cores	19	S	X	--	X	X	X	X	X	X	X	X
Bioaccumul-ative Effect												
M-1 to M-17	17	T	X	--	--	X	--	X	X	X	X	--
Background	1	T	X	--	--	--	--	X	X	X	X	--
Reference	2	T	X	--	--	--	--	X	X	X	X	--
Storm Water Toxicity												
ST1 to ST4	4	SW	--	X[g]	--	--	--	X	X	X	X	X
B-1 to B-4	4	BW	--	X[g]	--	--	--	X	X	X	X	X
Reference	1	BW	--	X[g]	--	--	--	--	--	--	--	--

a These numbers describe composited samples. The samples will be sub-sampled for screening of radioactivity, toxicity testing, physical testing, chemical analyses, or field and laboratory Quality Control (QC) samples
b Media Type: S = sediment, T = tissue, SW = storm water, BW = bay water
c Physical testing includes determination of grain size by ASTM Method D422
d Laboratory testing of radioactivity will be conducted on samples exhibiting radioactivity above background levels as determined by radioactivity screening. Radioactivity screening will include measurement of alpha and beta particles and gamma rays
e Analytical method: n-Pentyl Derivitization with Gas Chromatography/Flame Photometric Detection
f Toxicity testing of sediment samples involves the use of five replicates in 10-day solid phase bioassays and liquid suspended particulate phase bioassays
g Toxicity testing of storm and bay water samples involves a five dilution series
h Fecal coliform testing will be conducted by Fecal Coliform Filter Membrane Procedure (Standard Method 909C)

Table 1. Sampling and Analytical Program

Evaluation Program and Sample Location Numbers	Number of Samples[a]	Media Type[b]	Fecal Coliform Testing
Sediment Toxicity			
S-1 to S-17	17	S	---
Reference	3	S	---
Control	1	S	---
Sediment Cores	19	S	---
Bioaccumulative Effect			
M-1 to M-17	17	T	---
Background	1	T	---
Reference	2	T	---
Storm Water Toxicity			
ST1 to ST4	4	SW	X[h]
B-1 to B-4	4	BW	---
Reference	1	BW	---

a These numbers describe composited samples. The samples will be sub-sampled for screening of radioactivity, toxicity testing, physical testing, chemical analyses, or field and laboratory Quality Control (QC) samples
b Media Type: S = sediment, T = tissue, SW = storm water, BW = bay water
c Physical testing includes determination of grain size by ASTM Method D422
d Laboratory testing of radioactivity will be conducted on samples exhibiting radioactivity above background levels as determined by radioactivity screening. Radioactivity screening will include measurement of alpha and beta particles and gamma rays
e Analytical method: n-Pentyl Derivitization with Gas Chromatography/Flame Photometric Detection
f Toxicity testing of sediment samples involves the use of five replicates in 10-day solid phase bioassays and liquid suspended particulate phase bioassays
g Toxicity testing of storm and bay water samples involves a five dilution series
h Fecal coliform testing will be conducted by Fecal Coliform Filter Membrane Procedure (Standard Method 909C)

Table 2. Sample Handling Program

Sample Matrix and Analytes	Sample Volumes[a]	Sample Containers	Preservation Methods	Maximum Holding Times[b]
Sediment Samples				
VOCs[c]	10 grams	CAB tubes	Chill to 4° C	14 days
SOCs	10 grams	125 ml Glass jars or CAB tubes	Chill to 4° C	14 days/40 days
Metals	10 grams	125 ml Polyethylene jars or CAB tubes	Chill to 4° C	6 months (except Hg:28 days)
Pesticides & PCBs	100 grams	250 ml Glass jars or CAB tubes	Chill to 4° C	14 days/40 days
Tributyltin	10 grams	125 ml Polyethylene jars or CAB tubes	Freeze to ≤- 20° C	28 days
Radiation	10 grams	125 ml Glass jars or CAB tubes	Chill to 4° C	6 months
Physical: Grain Size	150 grams	1 liter plastic jar	Chill to 4° C	6 months
Total Organic Carbon	3 liters	1 liter Glass jars	Freeze to ≤- 20° C	6 months
Mussel Tissue Samples				
SOCs	10 grams	Heat Cleaned ZIPLOCK® bags with aluminum foil	Freeze to ≤- 20° C	14 days/40 days
Metals	10 grams	MICRO® detergent cleaned double ZIPLOCK® bags	Freeze to ≤- 20° C	6 months (except Hg: 28 days)
Pesticides & PCBs	10 grams	Hexane rinsed ZIPLOCK® bags with double aluminum foil	Freeze to ≤- 20° C	14 days/40days
Tributyltin	10 grams	MICRO® detergent cleaned double ZIPLOCK® bags	Freeze to ≤- 20° C	28 days
Radiation	10 grams	125 ml Widemouth plastic jars	Chill to 4° C	10 days
Storm Water Samples				
VOCs	40 mls	2-40 ml Glass bottles with teflon-lined caps	Chill to 4° C HCl to pH 2	14 days

Table 2. Sample Handling Program (continued)

Sample Matrix and Analytes	Sample Volumes[a]	Sample Containers	Preservation Methods	Maximum Holding Times[b]
SOCs	1 liter	2 liter Glass bottles	Chill to 4° C	7 days/40 days
Metals	200 mls	480 ml Polyethylene or Glass bottles	Chill to 4° C HNO_3 to pH <2	6 months (except Hg:28 days)
Pesticides & PCBs	1 liter	2 liter Glass bottles	Chill to 4° C	7 days/40 days
Tributyltin	1 liter	2 liter Polyethylene or Glass bottles	Chill to 4° C	28 days
Fecal Coliforms	100 mL	100 mL Glass bottles	Chill to 4° C	30 hours

a. These are the volumes required for analysis. To insure that the laboratory has sufficient amounts of sample, at least two times as much volume should be sent to the laboratory. Extra volume must also be provided for laboratory QC samples (matrix spike/matrix spike duplicate).
b. x days/y days = x is the extraction holding time, y is the holding time for analysis of the extracts
c. VOC analysis to be performed on sediment core samples only.
d. NA = Not applicable

Table 5. Analytical Methods for Storm Water Analyses

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
ST-1 - ST-4 B-1 - B-4	Water	CLP Inorganics	Aluminum	200.0
			Antimony	3.0
			Arsenic	10
			Barium	100.0
			Beryllium	5.0
			Cadmium	5.0
			Calcium	1000
			Chromium (total)	10.0
			Cobalt	50.0
			Copper	25
			Iron	100
			Lead (total)	3.0
			Magnesium	1000
			Manganese	15.0
			Mercury	0.5
			Molybdenum	10.0
			Nickel	40.0
			Potassium	1000
			Selenium	5.0
			Silver	10.0
			Sodium	1000
			Thallium	10.0
			Tin	40.0
			Vanadium	50.0
			Zinc	20.0
		CLP Pesticides/PCBs	alpha-BHC	0.05
			beta-BHC	0.05
			gamma-BHC (Lindane)	0.05
			delta-BHC	0.05
			Heptachlor	0.05
			Aldrin	0.05

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			Heptachlor epoxide	0.05
			Endosulfan I	0.1
			p,p'-DDE	0.1
			Dieldrin	0.1
			Endrin	0.1
			p,p'-DDD	0.1
			Endosulfan II	0.1
			p,p'-DDT	0.1
			Endrin aldehyde	0.1
			Endosulfan sulfate	0.1
			p,p'-Methoxychlor	0.5
			Endrin ketone	0.1
			Technical chlordane	0.5
			Toxaphene	1.0
			Aroclor 1016	0.5
			Aroclor 1221	0.5
			Aroclor 1232	0.5
			Aroclor 1242	0.5
			Aroclor 1248	0.5
			Aroclor 1254	1.0
			Aroclor 1260	1.0
		CLP SOCs	Phenol	10
			bis(2-Chloroethyl) Ether	10
			2-Chlorophenol	10
			1,3-Dichlorobenzene	10
			1,4-Dichlorobenzene	10
			Benzyl Alcohol	10
			1,2-Dichlorobenzene	10
			2-Methylphenol	10
			bis(2-Chloroisopropyl) Ether	10
			4-Methylphenol	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			N-Nitroso-di-n-Propylamine	10
			Hexachloroethane	10
			Nitrobenzene	10
			Isophorone	10
			2-Nitrophenol	10
			2,4-Dimethylphenol	10
			Benzoic Acid	50
			bis(2-Chloroethoxy)Methane	10
			2,4-Dichlorophenol	10
			1,2,4-Trichlorobenzene	10
			Naphthalene	10
			4-Chloroaniline	10
			Hexachlorobutadiene	10
			4-Chloro-3-Methylphenol	10
			2-Methylnaphthalene	10
			Hexachlorocyclopentadiene	10
			2,4,6-Trichlorophenol	10
			2,4,5-Trichlorophenol	50
			2-Chloronaphthalene	10
			2-Nitroaniline	50
			Dimethylphthalate	10
			Acenaphthylene	10
			3-Nitroaniline	50
			Acenaphthene	10
			2,4-Dinitrophenol	50
			4-Nitrophenol	50
			Dibenzofuran	10
			2,4-Dinitrotoluene	10
			2,6-Dinitrotoluene	10
			Diethylphthalate	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			4-Chlorophenyl-Phenyl Ether	10
			Fluorene	10
			4-Nitroaniline	50
			4,6-Dinitro-2-Methylphenol	10
			N-Nitrosodiphenylamine	10
			Azobenzene	10
			4-Bromophenyl-Phenyl Ether	10
			Hexachlorobenzene	10
			Pentachlorophenol	50
			Phenanthrene	10
			Anthracene	10
			Di-n-Butylphthalate	10
			Fluoranthene	10
			Benzidine	50
			Pyrene	10
			Butylbenzylphthalate	10
			3,3'-Dichlorobenzidine	20
			Benzo(a)Anthracene	10
			bis(2-Ethylhexl)phthalate	10
			Chrysene	10
			Di-n-Octylphthalate	10
			Benzo(b)Fluoranthene	10
			Benzo(k)Fluoranthene	10
			Benzo(a)Pyrene	10
			Indeno(1,2,3-cd)Pyrene	10
			Dibenz(a,h)Anthracene	10
			Benzo(g,h,i)Perylene	10
		GC/FPD[a] with n-pentyl-derivitization	Tributyltin	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
ST1-ST4	Water	CLP VOCs	Chloromethane	10
			Vinyl Chloride	10
			Bromomethane	10
			Chloroethane	10
			Trichlorofluoromethane	5
			1,1-Dichloroethene	5
			Trichlorotrifluoroethane	5
			Acetone	20
			Carbondisulfide	5
			Methylene Chloride	5
			trans-1,2-Dichloroethene	5
			1,1-Dichloroethane	5
			2-Butanone	20
			cis-1,2-Dichloroethene	5
			Chloroform	5
			1,1,1-Trichloroethane	5
			Carbon Tetrachloride	5
			Benzene	5
			1,2-Dichloroethane	5
			Trichloroethene	5
			1,2-Dichloropropane	5
			Bromodichloromethane	5
			2-Chloroethylvinyl Ether	5
			Vinyl Acetate	10
			trans-1,3-Dichloropropene	5
			4-Methyl-2-Pentanone	10
			Toluene	5
			cis-1,3-Dichloropropene	5
			1,1,2-Trichloroethane	5
			Tetrachloroethene	5
			2-Hexanone	10

Sample Numbers	Sample Matrix	Analytical Method	Analytical Constituents	Approximate Quantitation Limits (µg/L)
			Dibromochloromethane	5
			Chlorobenzene	5
			Ethylbenzene	5
			Total Xylenes	5
			Styrene	5
			Bromoform	5
			1,1,2,2-Tetrachloroethane	5
			1,3-Dichlorobenzene	5
			1,4-Dichlorobenzene	5
			1,2-Dichlorobenzene	5
ST1-ST4	Water	Fecal Coliform Filter Membrane Procedure	Fecal Coliforms	NA

a. Gas chromatography/flame photometric detection
NA - Not applicable

RESPONSE TO U.S. ENVIRONMENTAL PROTECTION AGENCY COMMENTS ON THE OCTOBER 27, 1991 ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN ADDENDUM FOR HUNTER'S POINT ANNEX

SPECIFIC COMMENTS:

Comment #1: The calculation for volume of seawater and volume of sediment to fill the test chamber to 950 mL should be clarified. As 775 mL to 175 mL does not represent a 4 to 1 ratio, what does the final sentence mean?

Response: Section 2.6.1.3, the "Introduction of Seawater and Sediments to Test Chambers" has been revised in the Environmental Sampling and Analysis Plan addendum.

Comment #2: Our biologist's experience with bioassays using mysid shrimp has found that there are no nets of sufficiently fine-mesh to remove mysid shrimp, pipets are generally used. If this turns out to be the case, this should be documented as a change when the results of the bioassay is reported.

Response: The following sentence has been added to the first bullet, Section 2.6.5.A.2 of the ESAP addendum: "If fine-mesh nets prove insufficient for removal of the mysid shrimp from the holding tanks, large diameter pipets will be used." The method used for removing mysid shrimp from the holding tank will be reported with the final bioassay results.

RESPONSE TO NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION COMMENTS ON THE OCTOBER 27, 1991 ENVIRONMENTAL SAMPLING AN ANALYSIS PLAN ADDENDUM FOR HUNTER'S POINT ANNEX

COMMENTS: Our comments over the addendum are, in general, the same as those already expressed by other agencies (e.g., CA EPA), that is, that the addendum completely reflect the agreements arrived at the 10 Sept. 1991 meeting.

Comment #1: The addendum does not reflect wholesale incorporation of the Swartz et al. (1985) protocol for static amphipod bioassays.

Response: **SWARTZ ET AL. METHODOLOGIES**

We acknowledge that the Environmental Sampling and Analysis Plan (ESAP) and Addendum do not reflect "wholesale incorporation of the Swartz et al., (1985) protocol for static amphipod bioassays". However, the deviations from the Swartz protocol in the ESAP are a result of comments by the involved agencies and subsequent revisions of the amphipod sediment bioassay protocol to reflect those comments. Several sections of the ESAP have been revised for better comformability with the Swartz et al., 1985 methods. However, changes that were made to the Swartz et al., protocol in response to agency requests remain as part of the ESAP amphipod bioassay protocol. The Navy has compared the procedures described in "Phoxocephalid Amphipod Bioassay for Marine Sediment Toxicity" by R.C. Swartz, W.A. DeBen, J.K.P. Jones, J.O. Lamberson, and F.A. Cole, 1985, against the amphipod bioassay procedures in the ESAP and addendum and have prepared a list of some of the differences in these procedures and the rationale for the deviations. In the following sections, the ESAP and addendum are referred to as "the ESAP".

1. BIOASSAY SPECIES

Species Selection:

Swartz et al. recommends the amphipod species Rhepoxynius abronius as the preferred species, although it states that other phoxocephalids or free-burrowing amphipods may be acceptable. Standard bioassay temperature and salinity for R. abronius are 15° C and 25 ppt salinity.

ESAP: At the January 10, 1991 Technical Review Committee meeting, the involved agencies were asked to select their species of choice for the proposed bioassays. Eohaustorius estuarius was selected by the agencies as

the amphipod species of choice for the solid-phase bioassay. The temperature and salinity proposed in the ESAP for the amphipod sediment bioassay is the same as the seawater at the collection site, as per the EPA/COE implementation manual protocol. Swartz et al., 1985 states that "if another species (other than R. abronius) is used, its temperature and salinity tolerance limits must be established to determine standard bioassay conditions". A salinity of 15 ppt for the bioassay seawater is tenatively proposed in the ESAP based on the paper "Measuring the Toxicity of Estuarine Sediment", 1989, by T.H. DeWitt, R.C. Swartz, and J.O. Lamberson which included discussions of the tolerance of E.estuarius to salinity, and a telephone conversation with J.O. Lanberson on the temperature and salinity tolerances of E. estuarius.

Field Collection:

ESAP: It has been proposed throughout the ESAP revisions that the amphipods to be used in the bioassays would be purchased from a commercial supplier.

Sorting:

The proposed sorting methods in the **ESAP** are the same as the **Swartz et al.** except for the temperature and salinity requirements which are different due to the different species to be used in the bioassays.

2. BIOASSAY SEDIMENT

Control Sediment:

The Swartz method for handling control sediments is included in Section 2.6.1.1 of the **ESAP** addendum. Control sediments will be press-sieved to remove macrobenthic organisms, as per agency requests and EPA/COE manual protocol, rather than wet-sieved as in **Swartz et al.** A provision has been added to the **ESAP**, however, for the adjustment of interstitial salinity, if it is necessary to avoid salinity stress on the organism, as suggested by **Swartz et al.**

3. BIOASSAY SEAWATER

The Swartz method recommends that the bioassay seawater be maintained at the salinity and temperature characteristic of the amphipod collection site and that salinity adjustments, if necessary be made with distilled water or with clean seawater. **Swartz et al.** recommends filtering the seawater.

The ESAP proposes to use artificial seawater of the same temperature and salinity as the amphipod collection site. The use of artificial seawater was added to the plan at the request of the U.S. EPA and NOAA. **The ESAP** proposes to filter the artificial seawater, prior to use, if a residue or precipitate is present after aging as agreed to by the agencies. Salinity adjustments will be made, if necessary, with distilled water or a brine prepared from distilled water and artificial sea salts (e.g. clean, artificial seawater).

4. BIOASSAY PROCEDURES

Swartz et al. procedures recommend that beakers be placed in a 15° C waterbath and be allowed to equilibrate overnight.

The ESAP proposes to place the beakers in a temperature controlled room to equilibrate.

The **Swartz method** suggests that amphipods be removed from the holding sediment by use of a 1.0 mm screen and transferred to sorting trays.

The ESAP proposes to use a 0.5 mm screen to remove amphipods from the sediment. The screen size was changed from a 1.0 mm screen to a 0.5 mm screen in response to an EPA request (EPA Comment #17 on the March 14, 1991 ESAP).

The Swartz method calls for the initial addition of water to the beakers to the 750 ml mark, the placement of the test beakers in a water bath to equilibrate, the addition of the test organisms, and then adding bioassay water up to the 950 ml mark.

The ESAP proposes to add bioassay water to the 750 ml mark, place the beakers in a temperature controlled room, add the control organisms when the water temperature has equilibrated, and finally, add bioassay water up to the 950 ml mark.

Monitoring:

The same conditions; pH, temperature, salinity, dissolved oxygen content, will be monitored in the **ESAP** as are suggested in the **Swartz method.** The monitoring of ammonia concentrations in each test chamber at the initiation and completion of the bioassays was added in response to agency requests.

Termination:

The Swartz method suggests a reburial phase at the termination of the bioassay to document the sublethal effects of toxicants on amphipod behavior.

The ESAP has no reburial phase as per the agreement with the agencies at the meeting on September 10, 1991.

5. EXPERIMENTAL DESIGN

Controls:

The Swartz method recommends that five replicate tests be run on sediments from a contaminant-free location.

The ESAP proposes to run five replicate tests on sediments from San Pablo Bay. This sampling location was agreed to by the agencies.

Response Criteria:

The Swartz method utilizes three response criteria: 1) emergence from sediment, 2) survival, and 3) the ability to rebury. However, **Swartz et al.** state that survival is the primary criterion of toxicity.

The ESAP proposes to use emergence from sediment and survival as response criterion; the reburial phase will not be included as agreed upon with the agencies at the September 10, 1991 meeting.

Field Design:

Swartz et al. state that biossay tests are usually not replicated from (test) stations to allow for maximum spatial coverage.

The ESAP proposes to run five replicate bioassay tests on test station samples to allow for statistical comparisons, as per EPA/COE "Greenbook", EPA requirements, and agency agreement.

Swartz et al. refers to the EPA/COE "Greenbook" for the application of statistical methods to bioassay data.

The ESAP proposes to use a combination of EPA/COE "Greenbook" and agency recommended statistical methods.

The Navy believes that there are no significant deviations from the Swartz method in the ESAP that would negatively affect the bioassay results. The ESAP bioassay was presented as a modified Swartz et al. method throughout the review process and the modifications that remain have been agreed to by the involved regulatory agencies.

Comment #2: Sediment samples should not be shipped in 15 liter containers; they should be shipped in such a fashion so as to exclude air and minimize overlying water. The requirement for a container greater than 10 liters was only to ensure homogeneity of mixing.

Response: The sediment sample compositing procedures and subsequent containerization of the samples, contained in the third paragraph on page 2 of the addendum, has been modified to include the use of smaller (2-liter) sample containers for storage and shipment of sediment samples for bioassay testing.

Comment #3: The addendum does not acknowledge use of reference toxicant test with the amphipod test or any other bioassays proposed.

Response: Reference toxicant tests will be conducted on each group of test organisms in order to determine the relative health and vigor of the organisms. As agreed upon at the September 10, 1991 meeting and as prescribed in the EPA/COE "Greenbook", the tests will be run without sediment. A description of the reference toxicant tests can be found in the revised addendum as follows:

Amphipod Sediment bioassays	**Section 2.6.1.2**
Modified Solid-Phase bioassays (*H. costata* and *N. caecoides*	**Section 2.6.2.3**
Liquid Suspended Particulate Phase bioassays:	
H. costata	**Section 2.6.5.A.4**
C. gigas* or *M. edulis	**Section 2.6.5.B.4**
C. stigmaes	**Section 2.6.5.C.4**

Comment #4: This discussion of stormwater sampling does not reflect the fact that sampling should cease when storm runoff ceases or when tidal back flow occurs.

Response: Flow reversal, as a general indicator of tidal backflow, has been added in Section 4.4.1 of the ESAP addendum as a condition for termination of storm water sampling.

RESPONSE TO DEPARTMENT OF TOXIC SUBSTANCES CONTROL COMMENTS ON THE OCTOBER 27, 1991 ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN ADDENDUM FOR HUNTER'S POINT ANNEX

SPECIFIC COMMENTS:

Comment #1: There was agreement that solid-phase amphipod bioassays would be conducted under the protocols outlined in Swartz, et al., (1985) as stated in the EPA/COE "Greenbook" (Section 11.2.1.3). The ESAP refers to several settling and decanting steps in "bullet item" three and "bullet item" five (Section 2.6.1.1) which are not amended in the draft addendum to the ESAP. These decanting steps are not present in the Swartz, et al. (1985) protocol and should be amended. Those sediments which can be pressure sieved should not require a settling period until the sediment is added to the test chamber.

Response: **In the revised "Addendum to the Environmental Sampling and Analysis Plan for Naval Station, Treasure Island, Hunters Point Annex, San Francisco, California" Section 2.6.1.1 has been revised to more accurately reflect the Swartz, et al. (1985) protocol. The sediment processing procedures involving settling and decanting steps will be used on the control sediment in the event that the interstitial salinity of the sediment is not within the tolerance limits of the test species, as outlined in the addendum. This protocol follows the Swartz et al. suggested methodology for control sediment processing. It is not anticipated that this step will be necessary, as the control sediment will be obtained from the same supplier as the test organisms, and the two should therefore be compatible.**

Comment #2: The third "bullet item" in Section 2.6.2.4 of the ESAP calls for each exposure beaker to "stand for at least 24 hours" prior to introduction of the test organisms. I cannot find this waiting period in the Swartz, et al. (1985) protocol. This settling period should be removed.

Response: **Section 2.6.2.4, on page 2-13 of the ESAP refers to the "Introduction of Seawater and Sediments to Test Chambers for the Modified Solid-Phase Bioassays" for the marine worm, Nephtys caecoides, and the mysid shrimp, Holmesiysis costata, not to the Amphipod Sediment Bioassay. The Swartz, et al. paper,"Phoxocephalid Amphipod Bioassay for Marine Sediment Toxicity", 1985, is designed for static bioassays using amphipods. The 24-hour sediment settling period procedure was taken from the EPA/COE "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual", 1991, Section 11.0, Guidance for Performing Biological-Effects Tests which was used for the preparation of the H. costata and N. caecoides bioassay procedures.**

Comment #3: There was agreement that short-term reference toxicant bioassays on each group of test organisms would be conducted without sediment in the exposure chambers (EPA/COE, Section 11.2.2). I can find no discussion of reference toxicant bioassays in either the ESAP or the addendum to the ESAP. Reference toxicant bioassays must be added to the ESAP addendum for all groups of organisms in all bioassays.

Response: The use of short-term reference toxicant bioassays on each group of test organisms to be conducted without sediment has been added to the ESAP in order to determine the relative health and vigor of the organisms used in the bioassays. The reference toxicant bioassay information can be found in the ESAP addendum as follows:

Amphipod Sediment bioassays	**Section 2.6.1.2,**
Modified Solid-Phase bioassays (H. costata and N. caecoides)	**Section 2.6.2.3,**
Liquid Suspended Particulate Phase bioassays:	
H. costata	**Section 2.6.5.A.4**
C. gigas or M. edulis	**Section 2.6.5.B.4**
C. stigmaes	**Section 2.6.5.C.4**

Comment #4: Section 2.4.1 of the addendum to the ESAP is somewhat confusing. How will "ten representative samples" (page 2, paragraph 3; referring to page 2-7 of the ESAP) consisting of a "one-liter aliquot of sediment" (page 2, paragraph 1; referring to page 2-7 of the ESAP) fill a "15 liter container"? The volume to be collected is not equal to the final volume of the container.

There was agreement on a procedure for covering the remaining composite sediment sample with water after removing subsamples from the composite sample container for chemical analysis. After considering this procedure in more detail we would suggest that the stored sediment sample completely fill the composite sample container during storage. This can be easily accomplished by mixing the individual sediment samples in an intermediate container until the color is uniform, removing the subsample necessary for chemical or physical analysis and completely filling a storage container for later use in the bioassays. This procedure will minimize any changes in sediment characteristic due to exposure to overlying water during storage.

Response: The sediment sample compositing procedures and subsequent containerization of samples, contained in the third paragraph on page 2 of the addendum has been modified for clarification. In addition, the use of smaller (2-liter) sample containers for storage and shipment of sediment samples for bioassays, has been added in response to DTSC's comment.

RESPONSE TO CALIFORNIA REGIONAL WATER QUALITY CONTROL BOARD COMMENTS ON THE OCTOBER 27, 1991 ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN ADDENDUM FOR HUNTER'S POINT ANNEX

Cover Letter: The staff of the San Francisco Bay Regional Water Quality Control Board (SFRWQCB) has completed its review of the above document dated November 1, 1991. Please note that at a TRC meeting on September 25, 1991, the importance of the addition of fecal coliform counts (methods to detect human waste in storm water) to the list of analyses, was discussed. The minutes of that meeting, listed as item XI, recorded the concurrence of the consultant to ensure the addition of this analysis to the work plans for both the storm drain study and the ESAP, yet this addition was not mentioned in the above addendum. Responses to the following comments shall be considered for incorporation into the ESAP addendum.

General Comments:

Comment #1: Will toxicity tests that "fail" because the control mortality is not within prescribed protocol limits be repeated? What provision is made for a sediment sample for which all of the controls of all of the toxicity tests fail to meet protocol limits. If tests are to be repeated, what quality assurance measures will be invoked to avoid exceeding holding time for the samples? What is the holding time for sediments to be used in this study?

Response: **Toxicity tests that "fail" because the control mortality is not within prescribed protocol limits ($\leq$10%) will be repeated. Sufficient sediment will be collected to run a second set of bioassay tests should the control mortality fall above 10 percent during the first test run. Sediments will be sent to the bioassay testing laboratory immediately (same day) following collection. The bioassay testing will commence the day after the samples are received. Although a holding time of 14 days is preferred, according to the EPA/COE "Evaluation of Dredged Material Proposed for Ocean Disposal - Testing Manual", 1991, Section 8.2.6.3, samples for biological testing should be tested within 2 weeks of collection, but the samples may be stored up to 6 weeks before testing commences, if necessary. As the test duration is ten days and the samples may be held up to 42 days, a second test set should be able to commence without exceeding holding times for the sediment. This information has been included in Section 2.4.1 of the ESAP addendum. It should noted that the ATT laboratory proposed to conduct the bioassays has been able to maintain control mortalities of less than 10 percent for bioassays of this type.**

Comment #2: Will reference toxicants be used for all toxicity tests?

Response: Reference toxicants will be run in conjunction with all toxicity tests in order to determine the relative health and vigor of the organisms used in the bioassays. The reference toxicant bioassay information can be found in the addendum as follows:

Amphipod Sediment bioassays	**Section 2.6.1.2**
Modified Solid-Phase bioassays (H. costata and N. caecoides	**Section 2.6.2.3**
Liquid Suspended Particulate Phase bioassays:	
H. costata	**Section 2.6.5.A.4**
C. gigas or M. edulis	**Section 2.6.5.B.4**
C.stigmaes	**Section 2.6.5.C.4**

Comment #3: The staff of the SFRWQCB recommends that some approach to determining whether the sewer and storm drain systems at HPA are separated be incorporated into the ESAP for storm water investigation. The staff of the SFRWQCB recommends that storm water be tested for human-specific viruses because this is the only known approach to distinguish human from other mammalian wastes. This approach requires the filtering of a large volume of storm water (at least 100 gal) through special filters. This type of testing is not widely performed, however, two potential alternative approaches are available.

The first alternative involves the testing of sediment from storm drains. Human enteric viruses can be cultured from sediment samples taken from the storm drains. This approach has the disadvantages of providing "false positives" when sediment contaminated with human waste from San Francisco Bay is transported into the storm drain system by tidal action. In addition, the viruses are persistent and remain in the sediments for periods of up to one month. Therefore, presence of human enteric viruses in the sediments may not be attributable to the most recent storm event. SFRWQCB staff has made contact with staff at the Orange County Sanitation District (OCSD) who would be willing to perform the sediment analyses for $320.00 per sample. SFRWQCB staff would be willing to oversee collection of the samples, perform the initial extractions and oversee the shipment of the extracted samples to OCSD staff.

The second alternative involves the use of biotechnology, currently under development at U.C. Irvine and due for release within 6 months. This technique involves the use of fluorescent DNA probes that "recognize" (bind to) unique DNA sequences on human-specific bacteria. Neither the costs nor whether this technique will be usable for both water and sediment samples is currently known.

Response: **As agreed upon at the September 25, 1991 TRC meeting, fecal coliform counts will be added to the storm water sample testing program. Fecal coliform counts will be estimated using standard methods for characterization of wastewater. Sampling and analysis of storm water for human-specific viruses is not proposed at this time for the following reasons:**

- **The primary objective of the ESAP is to assess the presence of chemical contamination in offshore areas of Hunters Point Annex.**
- **Testing for fecal coliform will be adequate as a screening tool to assess the possibility that connections between the systems exist.**
- **There are no standard methods for examining water for the presence of viruses. Available methodology has important limitations (Clesceri et al., 1989, Standard Methods for Examination of Water and Wastewater, 17th Edition) that are likely to compromise the usefulness of the results in interpreting possible system interconnections.**

With regard to sediment testing, collection of sediment samples from storm drains is not within the proposed scope of the ESAP. The Navy agrees that interpretation of data from sediment cultures would be equivocal given the persistence of human viruses, the possibility of transport and deposition of waste from the Bay into the drains, and the difficulty in attributing viral presence to the most recent storm event. Note that numerous POTW's discharge treated wastewater to the Bay that presumably contain some enteric viruses. Furthermore, the Water Quality Investigation of Storm Water Drainage (HLA, 1991) indicated that the stormdrain sampling locations proposed in the ESAP are tidally influenced and undoubtedly receive sediment from the Bay.

In summary, virus testing seems premature at this time. If fecal coliform testing suggests system interconnections, the need for further testing to distiguish between human and animal waste can be evaluated further. The Navy remains willing to cooperate and coordinate with the RWQCB if the RWQCB wishes to collect and analyze samples for human viruses.

Specific Comments:

Comment #1: p. 2-3, Section 2.2.2: There is no such word as "nontoxicity". The sentence should read, "...to support the assertion that the sediments are not toxic."

Response: On page 2-3, Section 2.2.2, the sentence at the end of the last paragraph is revised to read "If control sediment is purchased, the supplier will be required to furnish data to support the assertion that the sediments are not toxic." This change is reflected in the addendum.

Comment #2: p. 2-14, Section 2.6.2.7: The word "predated" means to date before the actual time. The correct word is "cannibalized".

Response: On page 2-14, Section 2.6.2.7, the last sentence of the second paragraph is modified to read "Organisms which are not recovered will be considered dead because once dead, organisms may decompose or be cannibalized." This change is reflected in the addendum.

Comment #3: Section 2.6.5.A.8, second sentence: delete reference to "sensitive parts" and change the sentence to, "any response to gentle probing, or gentle swirling of the water."

Response: In Section 2.6.5.A.8 of the addendum, the second sentence is modified to read "The organisms will be considered alive if they show any response to gentle probing or gentle swirling of the water."

Comment # 4: Section 2.6.5.A.10: What statistical tests will be used if the data fail to show homogeneity of sample variances?

Response: In the event that the liquid suspended particulate phase bioassay data fail to show homogeneity of variances, the t-test for unequal variances (Snedecor and Cochran, 1980) will be used. This information has been included in Sections 2.6.5.A.10, 2.6.5.B.10, and 2.6.5.C.10 of the ESAP addendum. The statistical analytical methods for the solid phase bioassays are described in the ESAP and addendum, Section 2.6.4.

Comment #5: Section 2.6.5.B.1, paragraph 2, second sentence: The units are g/kg/day. The correct units are parts per thousand per day salts (ppt/day) or g/L/day salts.

Response: In Section 2.6.5.B.1, paragraph 2 of the addendum, the second sentence is modified to read "Temperature will be changed at a rate not to exceed 2° C/day and salinity at a rate not to exceed 5 g/L/day salts."

Comment #6: Section 2.6.5.B.7: Strike the last part of the third sentence, after the word "tests". The fertilized embryo is not a feeding stage, therefore no food is necessary!

Response: In Section 2.6.5.B.7 of the addendum, the last sentence of the first paragraph is modified to read "The organisms will not be fed during the tests."

Comment #7: Section 2.6.5.B.9: Will reference toxicant tests be run for all test organisms, or only for the bivalve larvae test?

Response: Reference toxicant tests will be run for all test organisms. A description of these tests has been included in the addendum.

Comment # 8: Section 2.6.5.C.1: paragraph 3, last sentence: See above Comment #5.

Response: In Section 2.6.5.C.1 of the addendum, the last sentence of paragraph 3 is modified to read "Temperature will be changed at a rate not to exceed 2° C/day and salinity at a rate not to exceed 5 g/L/day salts."

Comment #9: Section 2.6.5.C.10: See above comment #4.

Response: In the event that the liquid suspended particulate phase bioassay data fail to show homogeneity of variances, the t-test for unequal variances (Snedecor and Cochran, 1980) will be used. This information has been included in Sections 2.6.5.A.10, 2.6.5.B.10, and 2.6.5.C.10 of the ESAP addendum. The statistical analytical methods for the solid phase bioassays are described in the ESAP and addendum, Section 2.6.4.

Comment # 10: p. 4-6, Section 4.6.2, fourth bullet: Strike the word "indigenous".

Response: On page 4-6, Section 4.6.2, the fourth bullet is changed to read "Sample water will be filtered with a plankton net to remove organisms." This change is reflected in the addedum.

Comment #11. p. 4-8, Section 4.9: Modify Tables 3 and 7 to include analyses of human enteric viruses.

Response: Fecal coliform testing will be added to Tables 3 and 7, however human enteric viruses will not be added pursuant to the discussion in the response to general comment #3.

ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN

DATED 31 JULY 1991

IS ENTERED IN THE DATABASE AND FILED AT ADMINISTRATIVE RECORD NO. **N00217.002268**

QUALITY ASSURANCE PROJECT PLAN FOR THE ENVIRONMENTAL SAMPLING AND ANALYSIS PLAN

DATED 31 JULY 1991

IS ENTERED IN THE DATABASE AND FILED AT ADMINISTRATIVE RECORD NO. **N00217.002269**